INFLUENCE OF AFLATOXIN B_1 ON GROWTH, PHOTOSYNTHETIC OXYGEN EVOLUTION AND REGREENING OF *CHLORELLA FUSCA* (CHOROCOCCALES, CHLOROPHYTA)

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ABSTRACT - Treatment of the green alga *Chlorella fusca* Shih. et Krauss with aflatoxin B_1 (AFB_1) induced a variety of toxic responses including cessation of cell division and inhibition of photosynthetic oxygen evolution. Other AFB_1-induced metabolic disorders involved inhibition of chlorophyll synthesis and reduction of cell contents of carbon, nitrogen, and phosphorus. Light-induced regreening of orange nitrogen-deficient cells was also inhibited.

KEY WORDS : Aflatoxin B_1, *Chlorella*, oxygen evolution, regreening.

INTRODUCTION

AFB_1 is a potent hepatocarcinogen known to induce toxic effects in animals that include inhibition of DNA-dependent RNA synthesis and changes in liver mitochondria (Sporn *et al.*, 1966). In plants, AFB_1 has been reported to inhibit germination and root growth of soybeans (Jones *et al.*, 1980), as well as death of floral buds (Reiss, 1969). Moreover, AFB_1 was shown to inhibit growth of *Chlorella pyrenoidosa* Chick (Sullivan & Ikawa, 1972), and chlorophyll synthesis in mung leaves (Sinha & Kumari, 1990). Reviews in the literature emphasize the need for work to shed more light on the effect of AFB_1 on the photosynthetic machinery (Dashek & Llewellyn, 1983). This paper describes the influence of AFB_1 on growth, and photosynthetic oxygen evolution in *Chlorella fusca* Shih. et Krauss which through its degreening-regreening capability provides an ideal model for examining the effects of AFB_1 on developing photosynthetic machinery.

MATERIALS AND METHODS

Chlorella fusca 211-15 from the Collection of Algal Cultures (Gottingen, Germany) was grown in 12th day/night cycles in a nitrate-rich medium (Grimme & Porra, 1974). Cells were degreened at 25°C and continuous illumination of 250 $\mu mol\ m^{-2}\ s^{-1}$ in a similar but nitrate-sparse medium (Grimme & Porra, 1974). For regreening, orange cells were harvested, washed and resus-

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Source : MNHN, Paris

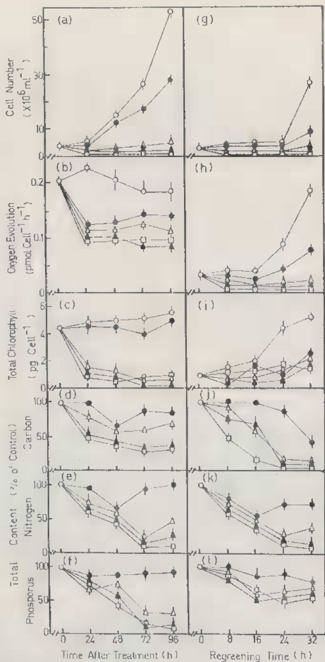

Fig. 1. - The effect of AFB_1 concentrations on growth and various physiological parameters of green (a-f) and orange nitrogen-deficient (g-l) cells of *Chlorella fusca*. (o) Control, (●) 0.1, (△) 1.0, (▲) 10, and (□) 100μml^{-1}. (± SE, n = 3). Control values (pg cell^{-1}) for green cells were 60 C, 5.3 N, 4.1 P, and for orange cells: 60 C, 5.4 N, 5.8 P.

Source : MNHN, Paris

pended in a nitrate-rich medium under continuous illumination. Cultures were sparged with fine bubbing at 25°C. AFB_1 in 0.03% dimethylsulfoxide was added to give concentrations in the range of 0.1-100 μgml^{-1}. Dimethylsufoxide (0.03%) was also added to control cultures. Cell numbers were determined with a haemocytometer (Richert-Jung, USA) and chlorophyll was determined after Boger (1964). Oxygen evolution was measured in an oxygen electrode (Rank Brothers, UK) at 25°C and a quantum flux density of 850μmol m^{-2} s^{-1}. For analysis, cells were harvested, washed and the contents of carbohydrates, nitrogen and phosphorus were determined after Dubois *et al.*, (1956), Vogel (1968) and Jackson (1960), respectively.

RESULTS

Treatment of green cells with 0.1μgml^{-1} resulted in 50% inhibition of growth with higher levels causing complete inhibition (Fig. 1a). Oxygen evolution was inhibited 24h after treatment, with high levels causing a marked inhibition and a reduction in chlorophyll content (Fig. 1b and c). Contents of carbon, nitrogen and phosphorus of treated cells gave reduced values when expressed as percentages of their corresponding control at each measurement. These values also exhibited declining trends in a pattern that was AFB_1-concentration dependent (Fig. 1d, e, and f, respectively).

Regreening of *Chlorella* cells resulted in a 14-fold increase in cell number which was inhibited 32% by 0.1 μgml^{-1} AFB_1 with higher levels causing complete inhibition (Fig. 1g). Regreening was complete within 24h as both oxygen evolution (Fig. 1h) and chlorophyll content (Fig. 1i) reached levels comparable to those of normal green cells (Fig. 1b, and c). Treatments resulted in further reduction of the residual oxygen evolving activity of the nitrogen-deficient cells (Fig. 1h) as well as complete inhibition of regreening (Fig. 1i). In addition, treatments resulted in reduction of cell contents of carbon, nitrogen, and phosphorus (Fig. 1j, k and l, respectively).

DISCUSSION

AFB_1 caused retardation of cell division, with high levels causing complete cessation of this process in both green and orange cells. The increase in cell number of control regreening cells indicated that each cell in the population had divided to give approximately eight daughter cells (Fig. 1g). Cells treated with 0.1 μml^{-1} attained a slower rate denoting some 75% inhibition of cell division and cells treated with higher levels failed to increase in number. AFB_1 is known to induce mitotic disturbances due to inhibition of chromatin-bound DNA-dependent RNA polymerase (Dashek & Llewellyn, 1983). Reduction of nitrogen contents of AFB_1-treated cells (Fig. 1e and k) may also reflect induced inhibition of protein synthesis as has previously been reported (Tripathi & Misra, 1981).

Oxygen evolution by green cells was inhibited by treatments with AFB_1. Inhibited photosynthesis is presumably largely responsible for the observed reduced carbon content. In orange cells AFB_1 caused reduction of the residual oxygen evolving activity and their failure to regreen. Chlorophyll synthesis and development of an oxygen evolving capability during regreening of orange nitrogen-deficient cells are considered to be two separable processes. The former being dependent on aerobic conditions, and the latter accompanies the regreening only in the light (Grimme, 1978). Inhibition of oxygen evolution in green

Source : MNHN, Paris

cells at 0.1 μgml^{-1} occurred without reduction of chlorophyll content (Fig. 1b and c) suggesting a direct inhibitory effect of AFB_1 on photosynthesis itself, possibly at the electron transport chain level.

ACKNOWLEDGEMENTS. - Thanks are due to Dr. M. Tantawy (University of Minia, Egypt) for donation of the culture. We are grateful to Dr. M.J. Earnshaw (University of Manchester, UK) for critically reading the manuscript.

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